Male palp organ morphology of three species of ground spiders (Araneae, Gnaphosidae)

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Abstract. A detailed morphological account of the male copulatory organs of three species of ground spiders, *Sergiolus capulatus*, *Herpyllus propinquus* and *Callilepis pluto* (Araneae, Gnaphosidae), is presented. The large sclerites (subtegulum, tegulum and embolus) appear to be homologous in all spiders. *Sergiolus* and *Zelanda* have a plesiomorphic palp organization. The increased complexity in the male bulb organization creates a locking mechanism that fixes the male palp position during intercourse in *Callilepis*, as well as in *Encoptarthria, Trachyzelotes* and *Zelotes*. The palp of *Herpyllus*, together with *Anzacia*, *Drassodes* and *Intruda*, demonstrates progressive modification of the male bulb.

Keywords: *Callilepis pluto*, ground-plan, *Herpyllus propinquus*, *Sergiolus capulatus*, tripartite male bulb

It is widely accepted that the male palp specifically fits into the female epigynum of the same species. The importance of male and female genitalia in species identification has long been recognized, since it was first used for this purpose (Westring 1861, Menge 1866, Wagner 1886, 1888, Engelhardt 1910, Comstock 1910, 1912).

Studies of male palp morphology show that the plesiomorphic state for this organ is characterized by a tripartite organization (Haupt 1983, Kraus 1978, 1984, Szombathy 1915). This type of palp consists of three basic sclerites – a subtegulum, tegulum and embolus – connected by three inflatable membranes: the basal, medial, and embolar hematodochae. This type of male palp organization was termed "hydraulic", in contrast to the other type of the male palp he termed "glandular" (Kraus 1978, 1984). The latter is characterized by the progressive fusion of all three sclerites into one capsule, accompanied by complete reduction of the membranes and two bulb muscles. This progressive reduction of sclerites, membranes and muscles evolved several times (Kraus 1978, 1984, Huber 1994).

Gnaphosid spiders can be divided into three major groups according to their male palp organization (Zakharov & Ovtcharenko 2011). The closest condition to the ancestral type of male palp was observed in *Zelanda erebus* (L. Koch, 1873); a peculiar species from New Zealand. *Drassodes lapidosus* (Walckenaer, 1802), *Intruda signata* (Hogg, 1900), and *Anzacia gemmea* (Dalmas, 1917) demonstrate significant "simplification" in the male palp construction. The embolic division of these spiders tends to fuse with the tegulum and, thus, transforms the palp into an essentially bipartite structure. By contrast, spiders of the genus *Encoptarthria* Main, 1954 demonstrate an increase in the complexity of male palp organization (Ovtcharenko & Zakharov 2007). Their palp contains an additional sclerite positioned between the tegulum and embolus, which probably functions as a flexible bridge and facilitates movement between the tegulum and embolus. This present study continues the morphological study of the expanded male palps of the ground spiders (cf. Zakharov & Ovtcharenko 2011).

Materials and methods

Genital bulbs of the following species – which represent two groups of gnaphosid spiders (the *Laronius* and *Herpyllus* groups) (Murphy 2007) – were studied: *Callilepis pluto* Banks, 1896, *Herpyllus propinquus* (Keyserling, 1887) and *Sergiolus capulatus* (Walckenaer, 1837). All three species were collected as follows: USA, Black Rock Forest, Cornwall, NY, 41°24'29"N 74°01'18"W, June 1999, leg. A. Tanasevitch and V. Ovtcharenko, coll V. Ovtcharenko.

The male palps of ground spiders for this study were prepared using a standard procedure (Comstock 1910, Sierwald 1990). The left palps were detached

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and submerged overnight in a weak watery solution of potassium hydroxide (KOH). It makes the bulb expend to various degrees. The bulb was then transferred into distilled water, where it continued to inflate. All prepared palps were preserved in 75 % alcohol. Drawings were made with the aid of a dissecting microscope (Nikon SMZ-U). Drawings were scanned and corrected with the computer program, Adobe Photoshop Lightroom 4.

Recent study on recognising homology status demonstrates that homology based on topology is the best criterion for male palp structures. A special similarity is close to that of topology and each criterion is better and contains fewer violations than homology based strictly on function (Agnarsson & Coddington 2008). This study supports the traditional view, and topology still remains the most reliable criterion of homology. For this reason, in order to reach a decision on the homologous status of a particular structure of the bulb, the following classical and widely applied criteria were used: 1) position of the structure; 2) morphological similarity with other known structures; 3) correspondence of the structure with other characteristics (Remane 1956, Patterson 1982, Coddington 1990, Sierwald 1990).

As noted above, it is generally accepted that the tripartite genital bulb in male spiders is a plesiomorphic characteristic (Platnick & Gertsch 1976, Kraus 1978, Haupt 1983, Sierwald 1990). This present study supports the conclusion that the large sclerites (subtegulum and tegulum) are homologous across all spiders (see also Kraus 1978, Coddington 1990, Sierwald 1990). These sclerites are organized around a tube. This tube has an enlarged, closed end (fundus), a long coiled tube (sperm duct), and a narrow tube with an opening at the end (ejaculatory duct) (Comstock 1910, 1912). This tube serves as a temporary sperm reservoir. Before mating, males fill their palps with sperm, which is stored here until mating occurs.

The terms proximal and distal here refer to the position of a structure in relation to the trajectory of the sperm duct. The structures that occupy a position close to the fundus are considered proximally located. On the other hand, the structures that are close to the ejaculatory duct are referred to here as distal.

The terms median apophysis and conductor are used to name tegular apophyses that are supposed to be homologous within all gnaphosoids. An inflatable membranous projection on the upper surface of the first half of the tegulum – which is merely an outgrowth of the membranous walls of the tegulum and is closely related to the tip of the embolus – is labeled the conductor. The median apophysis is a heavy sclerotized structure that occupies a position more distal on the tegulum than the conductor. It connects to the tegulum via an inflatable membrane, and does not directly associate with the embolus. The embolic division of the bulb is identified by the constriction of the sperm duct and its transformation into the narrow ejaculatory duct. According to this definition of the embolic division, the distal sclerotized tube is assigned to the embolic bulb division (Sierwald 1990).

Results

The retrolateral tibial apophysis in ***Sergiolus capulatus* (Walckenaer, 1837)** is a simple, massive, hooked structure (Fig. 1). The basal and median hematodochae are well developed. The subtegulum and tegulum are open spirals with a single loop. The median apophysis and conductor are absent. The embolus is short, curved clock-wise, and grooved. The area of the embolus, close to its tip, has a membrane (Fig. 1, TM) which is associated with the embolus. The function of this membrane is unknown; it probably plays a supporting role during copulation. This membrane does not connect to the tegulum. Instead it is a membranous outgrowth of the base of the embolus. Thus, taking into account the topological criterion of homology (Agnarsson & Coddington 2008), it cannot be regarded as a conductor, and we refer to it as a "terminal membrane". Proximally the embolus is attached to the distal tubular membrane, which connects it to the distal part of the tegulum.

In general, the bulb of the *Sergiolus* is very simple, tripartite, has three well-separated major sclerites (subtegulum, tegulum, and embolus), and lacks the median apophysis and conductor.

The retrolateral tibial apophysis of ***Herpyllus propinquus* (Keyserling, 1887)** is short, broad, slightly curved, and sharp at its tip (Fig. 2). The basal and medial hematodochae are well developed. The subtegulum and tegulum are heavily sclerotized rings. The median apophysis is a simple hook. The conductor is a comparatively simple, inflatable membrane. At its tip this membrane is divided into two lobes. The embolus is comparatively short. The proximal part of the embolus is broad and fuses with the tegulum. Its distal part is short, slender, hook-like, and rests in the groove between the top lobes of the conductor.

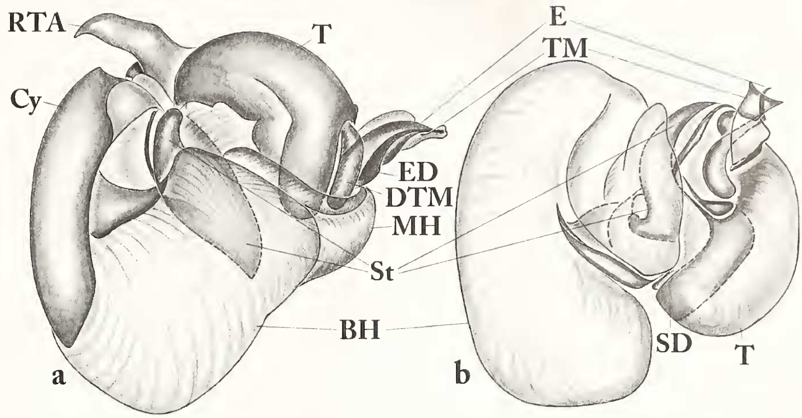

Fig. 1a-b: *Sergiolus capulatus*, left palp; a. Ventral view; b. Lateral view. BH – basal hematodocha; Cy – cymbium; DTM – distal tubular membrane; E – embolus; ED – ejaculatory duct; MH – median hematodocha; RTA – retrolateral tibial apophysis; SD – sperm duct; St – subtegulum; T – tegulum; TM – terminal membrane.

In conclusion, the bulb of *Herpyllus* is among the most modified male reproductive organs seen in the ground spiders. The fusion of the embolus with the tegulum transforms the male bulb of these spiders into a bipartite structure.

The male of ***Callilepis pluto* Banks, 1896** does not have a retrolateral tibial apophysis (Fig. 3). The cymbium of these spiders is very characteristic. It is flattened; its length is two times longer than its width and somewhat spoon-shaped. The bulbus is well-developed. A basal hematodocha connects the subtegulum to the petiolus and the alveolus of the cymbium. The median hematodocha connects the tegulum and subtegulum. There is a distal tubular membrane between the tegulum and embolus that binds them through the distal tegular projection on one side and the broad base of the embolus on the other side.

The median apophysis is attached to the tegulum via a flexible membrane, approximately two-thirds of the way along the ventral part of the tegular ring. The insertion of the median apophysis on the tegulum is closer to the embolic division than the insertion of the conductor. The shape of the median apophysis is very unusual. It is divided into two large, massive, and heavily sclerotized hooks. The conductor is a small, weakly-developed outgrowth of the membrane that covers the tegulum.

The embolic part of the bulb is connected to the tegulum by the distal tubular membrane. This flexible membrane permits the embolus to rotate around

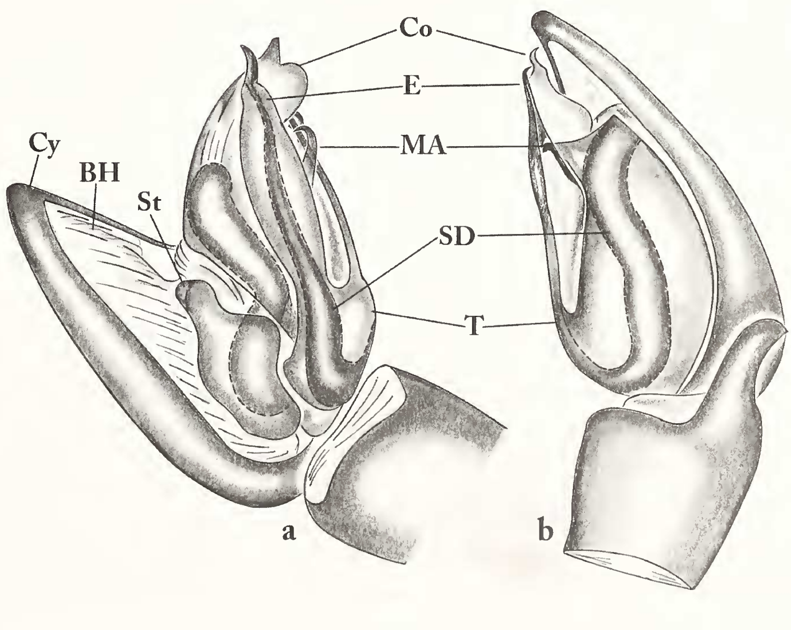

Fig. 2a-b: *Herpyllus propinquus*, left palp; a. Prolateral view; b. Retrolateral view. BH – basal hematodocha; Co – conductor; Cy – cymbium; E – embolus; MA – median apophysis; SD – sperm duct; St – subtegulum; T – tegulum.

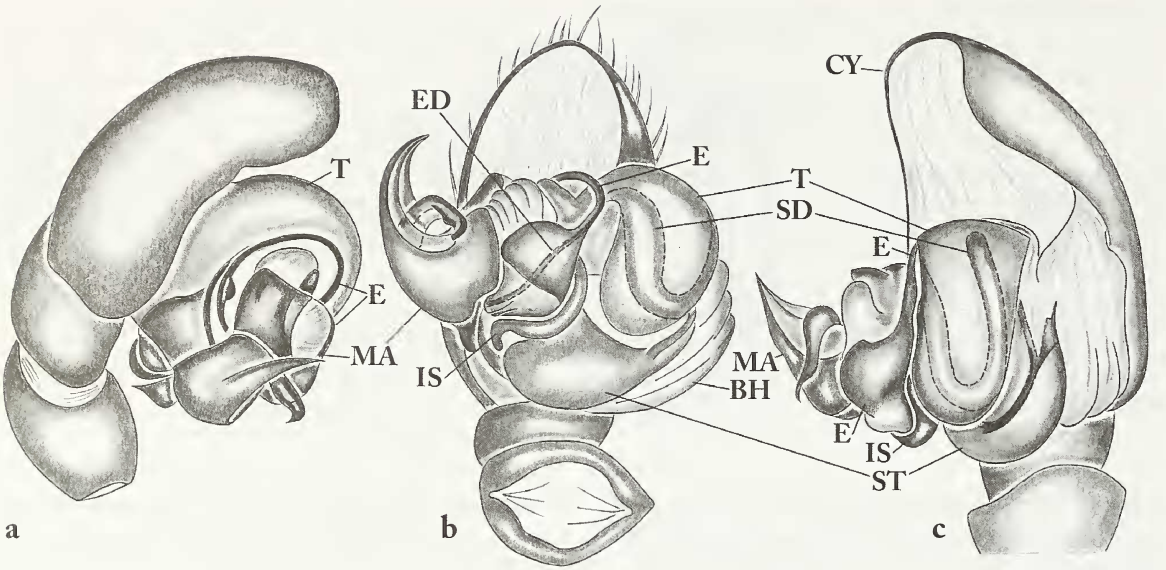

Fig. 3a-c: *Callilepis pluto*; left palp; a. Antero-retrolateral view; b. Ventral view; c. Prolateral view. BH – basal hematodocha; Cy – cymbium; E – embolus; ED – ejaculatory duct; IS – intercalary sclerite;MA – median apophysis; SD – sperm duct; St – subtegulum; T – tegulum.

the distal tegular projection as if it were an axis. The embolus is very long, slender, slightly flattened and semi-circularly curved. It has a broad basal part and bulged outgrowths on its distal part (embolar distal projection). The most peculiar structure found in the bulb of this species is a long, narrow sclerite between the tegulum and the embolus (Fig. 3, IS). Its position, special relations with other sclerites of the bulb and function are similar to the intercalary sclerite found in *Zelotes* (Platnick & Shadab 1983). It allows us to term this structure an "intercalary sclerite" here too. Probably, this additional sclerite increases the mobility of the embolus during copulation, as was described for *Zelotes* (Senglet 2004, 2011, 2012).

Discussion

Analysis of the present material allows us to further develop an understanding of the organization of the male palp in gnaphosid spiders. This study supports the previous observation that there are three basic ground-plans in gnaphosid male bulb morphology (Zakharov & Ovtcharenko 2011). The tripartite genital bulb in male spiders is a plesiomorphic characteristic (Platnick & Gertsch 1976, Kraus 1978, 1984, Haupt 1983, Coddington 1990, Sierwald 1990) and includes three basic sclerites: a subtegulum, tegulum and embolus that are bound together by inflatable membranes. These large sclerites (subtegulum, tegulum and embolus) of all Entelegynae are homologous. The basic hematodocha – the membrane that attaches the subtegulum to the alveolus of the cymbium and the median hematodocha that binds the subtegulum and tegulum – are also homologous across all Entelegynae. The use of the term "terminal hematodocha" (Comstock 1910, 1912) should be avoided because its description and position in the bulb has not been clearly identified. Instead, the term "distal tubular membrane" is used here for the membrane that connects the distal part of the tegulum to the proximal end of the distal sclerotized tube or embolus. The term "terminal membrane" is proposed for the membranous outgrowth of the embolus.

Among ground spiders, the bulbs of *Sergiolus* and *Zelanda* are closest in morphology to that of the ancestral male palp. All major sclerites and membranes are present in the male bulbs of these spiders. Additionally, some derived palp forms have increased bulb complexity. The ground spiders of the genus *Callilepis*, as well as *Encoptarthria*, *Zelotes* and *Trachyzelotes* (Miller 1967, Platnick & Shadab 1983, Senglet 2004, 2011, 2012, Zakharov & Ovtcharenko 2011), have additional sclerites in the embolic division. They have a distal sclerotized tube (in *Encoptarthria*) or an intercalary sclerite (in *Callilepis* and *Zelotes*) between the tegulum and embolus that are flexibly connected to each other. This additional sclerite increases the mobility of the embolic part of the bulb. Furthermore, on the embolic part of the bulb, these spiders have subterminal and terminal apophyses. The presence of a number of additional sclerites in the embolar part suggests that increased complexity in the male bulb organization creates a locking mechanism during intercourse in these spiders (Sierwald & Coddington 1988, Huber 1994).

Herpyllus, together with the genera *Anzacia*, *Drassodes* and *Intruda* (Zakharov & Ovtcharenko 2011), represents progressive reduction of the number of sclerites in the male bulb. The proximal part of the embolus and the distal end of the tegulum in these spiders are fused. Thus, the embolus in these spiders is firmly attached to the tegulum and the distal tubular membrane completely disappears. The other major sclerites of the bulb in spiders of these genera are also significantly reduced.

Male bulb evolution in ground spiders was not linear, and analogous structures may appear independently in different groups. Such parallel evolution of the male palp – characterized by a secondary simplification through fusion of the apical and median bulbus sclerites and simultaneous reduction of the extensible membrane – has occurred independently in many groups of spiders. It was observed in orthognath (e.g. Theraphosidae), haplogyne (Sicariidae, Scytodidae, Pholcidae), and entelegyne spiders (*Castianeira*, Corinnidae) (Kraus 1984, Huber 1994). These observations suggest that there is a tendency in male spider palp evolution for the "pyriform male palp organ" to develop into the "glandular bulb" (Kraus 1984). Gnaphosid spiders also follow this major evolutionary trend, by which the embolus of their bulb fuses with the tegulum and thus changes the tripartite male palp into the bipartite. This process took place independently many times in different groups of the gnaphosid spiders. We observe this in the present study in *Herpyllus*, and it was previously seen in *Drassodes* and *Intruda* (Zakharov & Ovtcharenko 2011). Another phenomenon of the parallel transformation of the male bulb happens with those ground spiders whose male palpal organ undergoes an increase of external construction complexity. Thus, we have found the intercalary sclerite in the bulb of *Callilepis pluto*. The topology, special similarity, and function of this sclerite are the same as the intercalary sclerite in *Zelotes*. However, these two genera belong to different subfamilies of gnaphosid spiders and imply that they may have developed independently.

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